ISSN 1067-4136, Russian Journal of Ecology, 2013, Vol. 44, No. 3, pp. 231–234. © Pleiades Publishing, Ltd., 2013.
Original Russian Text © S.Zh. Kolumbaeva, D.A. Begimbetova, A.V. Lovinskaya, A.M. Kalimagambetov, 2013, published in Ekologiya, 2013, No. 3, pp. 216–220.

Studies on the Yellow Ground Squirrel (*Citellus fulvus* Licht.) in Biotopes Contaminated with Phenylpyrazoles

S. Zh. Kolumbaeva, D. A. Begimbetova, A. V. Lovinskaya, and A. M. Kalimagambetov

Al-Farabi Kazakh National University, pr. Al-Farabi 71, Almaty, 050040 Kazakhstan;
e-mail: S_kolumb@mail.ru

Received August 29, 2011

Abstract—The presence of fipronil and fipronil-sulfone has been revealed in components of natural ecosystems affected by application of fipronil-based pesticides. It has been found that ground squirrels *Citellus fulvus* from biotopes contaminated with phenylpyrazoles are characterized by a high LPO level as well as by cytogenetic instability manifested in the increased frequency of structural and genomic mutations. Chromatid rearrangements prevail in the spectrum of chromosome aberrations, which indicates that a chemical mutagen is present in the environment.

Keywords: Yellow ground squirrel *Citellus fulvus*, phenylpyrazoles, fipronil. fipronil-sulfone, bone marrow cells, mutagen, chromosome aberrations, lipid peroxidation (LPO), South Kazakhstan Region

DOI: 10.1134/S1067413612060069

The development of intensive agriculture requires the production and application of new pesticides, most of which have mutagenic activity (González et al., 2003; Losi-Guembarovski, 2004). According to WHO data, pesticides occurring in food products account for 1% of poisoning cases annually recorded worldwide. Their toxicity affects not only target species but also has an effect on many other organisms, including natural predators and parasites of these species. Moreover, application of pesticides results in the development of pest populations resistant to them and has adverse consequences for the genomes of other species (Biolognesi, 2003; Mukhopadhyay, 2004). However, their use is inevitable as long as it is economically profitable.

In recent years, second-generation phenylpyrazole insecticides containing fipronil as the active ingredient have been extensively used in different regions of Kazakhstan to control locusts and other pests (Sagitov, Ysak, and Evdokimov, 2002). Fipronil has been shown to undergo biological transformation in the natural environment and the body of mammals, with its metabolites being more toxic and persistent than the initial substance (Fung et al., 2003). The results of experiments on white mongrel rats provide evidence that fipronil and its metabolite fipronil-sulfone accumulate in visceral organs and have distinct toxic and genotoxic properties (Begimbetova et al., 2009, 2010). Therefore, we decided to study the contents of phenylpyrazoles in environmental objects and perform cytogenetic analysis in the yellow ground squirrel (*Citellus fulvus*), a common rodent species, from the rangelands of South Kazakhstan Region treated with fipronil-based insecticides.

MATERIAL AND METHODS

Soil and plant samples, locusts, and ground squirrels were collected in Arys, Shardara, and Kazygurt districts of South Kazakhstan Region (the last district was a conditional control area). Five male ground squirrels from each district were included in analysis. The animals were sacrificed under Nembutal anesthesia to collect bone marrow from the tibia for cytological analysis. Preparations were made and stained with azure–eosin according to the standard Romanovskii–Giemsa procedure (Grafodatskii and Radzhabli, 1988). Metaphase cells were analyzed for the frequency and spectrum of chromosome aberrations and photographed under an Axioskop-40 light microscope (Carl Zeiss, Germany).

Biochemical analysis for LPO was performed using standard methods to measure the contents of lipid hydroperoxides (Gavrilov and Mishkorudnaya, 1983) and malonic dialdehyde (MDA) (1977). The contents of fipronil and fipronil-sulfone were measured by gas–liquid chromatography according to the methodological guidelines approved by the RF Chief Public Health Physician (*Opredelenie...*, 2003).

The results were processed statistically using Student's *t*-test (Rokitskii, 1978).

RESULTS AND DISCUSSION

Many organic pesticides, including fipronil and its metabolites, can accumulate in the soil, plants, and

Table 1. Contents of phenylpyrazoles in components of natural ecosystems affected by application of fipronil-based pesticides

Sampling area	Fipronil content, mg/kg		
	soil	plants	locusts
Arys district	0.074 ± 0.007	0.037 ± 0.004	0.097 ± 0.010
Shardara district	0.090 ± 0.009	0.056 ± 0.005	0.078 ± 0.010
Kazygurt district (conditional control area)	–	–	–
Sampling area	Fipronil-sulfone content, mg/kg		
Arys district	0.045 ± 0.006	0.007 ± 0.001	0.040 ± 0.005
Shardara district	0.057 ± 0.010	0.005 ± 0.001	0.040 ± 0.006
Kazygurt district (conditional control area)	–	–	–

Table 2. Contents of phenylpyrazoles in organs of *Citellus fulvus* ground squirrels from contaminated biotopes

Sampling area	Fipronil content, mg/kg		
	liver	kidney	muscles
Arys district	0.0030 ± 0.0001	0.0010 ± 0.0001	0.0001 ± 0.0001
Shardara district	0.0030 ± 0.0001	0.0010 ± 0.0001	0.0004 ± 0.0001
Kazygurt district (conditional control area)	–	–	–
Sampling area	Fipronil-sulfone content, mg/kg		
Arys district	0.0040 ± 0.0001	0.0010 ± 0.0001	0.0005 ± 0.0001
Shardara district	0.0050 ± 0.0001	0.0020 ± 0.0001	0.0001 ± 0.0001
Kazygurt district (conditional control area)	–	–	–

animal bodies. For this reason, we studied the contents of phenylpyrazoles in the soil, plants, locusts, and ground squirrels from areas affected by fipronil (Table 1). The results showed that soil samples from the rangelands of Arys and Shardara districts treated with Adonis insecticide contained fipronil in amounts exceeding the maximum allowable concentration (MAC) by factors of 1.5 and 1.8, respectively, although the rate of its field application against pest insects remained within the norm (*Spravochnik*..., 2008). Plants from these rangelands also contained fipronil and its metabolite, fipronil-sulfone, while no traces of these xenobiotics were detected in control plants from Kazygurt district. Fipronil in the environment is partly converted into fipronil-sulfone as a results of oxidation reactions in the soil and plants. Such conversion also occurred in locusts, since fipronil-sulfone was found in these insects. Locusts collected in the conditionally clean territory of Kazygurt district contained only traces of these xenobiotics.

The results of analysis for the contents of fipronil and fipronil-sulfone in *C. fulvus* ground squirrels are shown in Table 2. Both these compounds were found in the visceral organs of rodents, with the amount of fipronil-sulfone in the liver exceeding that of fipronil. Our previous experiments on laboratory rats treated with fipronil showed that its contents in visceral organs decreased with time against the background of increase in the contents of fipronil-sulfone (Begimbetova et al., 2010). The results of this study provide evidence that fipronil is metabolized in *C. fulvus* ground squirrels, with fipronil-sulfone as metabolic products being accumulated in their organs.

Cytogenetic analysis of *C. fulvus* bone marrow (Table 3) showed that the frequencies of aberrant and polyploid cells in control animals from Kazygurt district were 2.44 and 0.19%, respectively, and the number of aberrations per 100 metaphases averaged 2.62. Their spectrum included aberrations of both chromosome (double terminal deletions) and chromatid types (single terminal deletions and dot fragments). These results agree with data on the background frequency of aberrant cells (about 2–3%) determined for small mammal populations from conditionally clean areas (Gileva et al., 1992; Dmitriev, 1997).

Comparative analysis of test parameters in animals from the control area and contaminated biotopes of

Table 3. Spectrum and frequencies of chromosome aberrations revealed in bone marrow cells of *Citellus fulvus* ground squirrels from biotopes of Southern Kazakhstan oblast affected by application of fipronil-based pesticides

Sampling area	Number of metaphases studied	Aberrant cell frequency ($M \pm m$), %	Number of aberrations per 100 metaphases			Polyploid cells, %
			total	chromosome aberrations	chromatid aberrations	
Kazygurt district (control)	1028	2.44 ± 0.49	2.62 ± 0.41	0.29 ± 0.12	2.33 ± 0.45	0.19 ± 0.12
Arys district	814	6.18 ± 1.03*	7.20 ± 1.14**	0.76 ± 0.25	6.44 ± 0.31***	1.47 ± 0.30**
Shardara district	921	7.25 ± 1.18**	8.47 ± 1.32**	0.82 ± 0.40	7.65 ± 1.04**	1.68 ± 0.49*

Note: Asterisks indicate that differences from the control are significant at * $p < 0.05$, ** $p < 0.01$, or *** $p < 0.001$.

Arys district showed that the frequency of structural and genomic mutations was significantly higher in the latter group. In particular, the frequency of aberrant cells in the bone marrow was higher by a factor of 2.5 ($p < 0.05$), and the number of aberrations per 100 metaphases, by a factor of 2.7 ($p < 0.01$). Their spectrum also included both chromosome and chromatid aberrations.

A similar pattern of chromosomal instability was observed in ground squirrels from Shardara district. Compared to the control group, the total frequency of aberrant cells was higher by a factor of 3.0 ($p < 0.01$); the number of aberrations per 100 metaphases, by a factor of 3.2 ($p < 0.01$); and the frequency of polyploid metaphases, by a factor of 8.8 ($p < 0.05$). Both chromosome and chromatid aberrations were recorded.

Comparative analysis of aberration spectra in ground squirrels from contaminated and control biotopes showed that the significant increase in aberrant cell frequency and the number of aberrations per 100 metaphases in the former was accounted for mainly by chromatid aberrations. The above parameters in animals from Arys and Shardara districts were 2.8 and 3.3 times higher than in the control group ($p < 0.001$ and $p < 0.01$, respectively), while statistically significant differences between the Arys and Shardara groups were absent.

Histological analysis of the liver in rats poisoned with fipronil under laboratory conditions provided evidence for adipose degeneration of this organ, with consequent damage to cell membranes and intensification of LPO processes (Begimbetova et al., 2010). Biochemical analysis for primary and secondary LPO products in ground squirrels from biotopes affected by phenylpyrazoles revealed a similar picture (Table 4). Compared to the control group, the contents of lipid peroxides and MDA were increased 1.5-fold ($p < 0.01$) in animals from Arys district and 1.6-fold ($p < 0.05$) in animals from Shardara district, in the absence of significant differences between these two groups.

In nature, mutations in somatic cells can spontaneously arise in any individual (Gileva et al., 1992; Dmitriev, 1997). Most cells with unbalanced chromosomal alterations are either unviable and die off or are eliminated by the immune system. The frequency of such disturbances may markedly increase under the effect of mutagenic factors. Genotoxic agents inhibit the system of DNA repair and the immunes system, which leads to disturbances in cytogenetic homeostasis and accumulation of cells with chromosome aberrations.

Cytogenetic studies on representatives of a common rodent species from biotopes affected by fipronil-based insecticides show that the frequency of structural and genomic mutations in these animals is higher than in animals from the conditional control area. The increase in the frequency of aberrant cells and the number of aberrations per 100 metaphases is explained mainly by induction of chromatid aberrations. An elevated level of such aberrations in rodents captured in nature is evidence for chemical contamination of the territory they inhabit.

Thus, the results of cytogenetic, chromatographic, and biochemical analyses show that the state of natural ecosystems subjected to application of fipronil-based pesticides is unfavorable. In addition, cytogenetic data provide evidence for increasing genetic load in natural

Table 4. Contents of lipid hydroperoxides (LHP) and malonic dialdehyde (MDA) in the livers of *Citellus fulvus* ground squirrels from biotopes affected by application of fipronil-based pesticides

Sampling area	Contents, mmol/mg	
	LHP	MDA
Kazygurt district (conditional control area)	3.53 ± 0.27	4.06 ± 0.43
Arys district	5.39 ± 0.41**	6.13 ± 0.31**
Shardara district	5.57 ± 0.74*	6.95 ± 0.55**

Note: Asterisks indicate that differences from the control are significant at * $p < 0.05$ or ** $p < 0.01$.

rodent populations inhabiting these ecosystems. The acceleration in mutation rate leads to an increase in the number of individuals with congenital defects, and this genetic load may threaten the survival of the population (Dubinin, 2000; Altukhov, 2004). Cytogenetic and biochemical defects observed in *C. fulvus* ground squirrels are indicative of the presence of genotoxic factors in their natural environment and also of changes in the general health status of individuals in mammal populations inhabiting the study region.

ACKNOWLEDGMENTS

This study was supported by the Science Foundation of the Republic of Kazakhstan, project no. GR-0109RK00153 (2009–2011).

REFERENCES

Altukhov, Yu.P., Dynamics of Gene Pools under Anthropogenic Impact, *Vestn. Vses. O–va Genet. Selekts.*, 204, vol. 8, no. 2, pp. 40–59.

Begimbetova, D.A., Kolumbaeva, S.Zh., Kalimagambetov, A.M., et al., Genotoxic Effects of Fipronil on Rats of Different Ages, *Vestn. Kazakh. Nats. Univ., Ser. Biol.*, 2009, no. 1 (40), pp. 78–83.

Begimbetova, D.A., Kolumbaeva, S.Zh., Kalimagambetov, A.M., et al., Effects of Phenylpyrazole Pesticides on Mammals, *Nauka i kul'tura v globalizovannom mire: Mat-ly 3-i mezhdunarodnoi mezhdistsiplinaronoi konferentsii Gumbol'dt-Kolleg v Kyrgyzstane* (Science and Culture in a Globalized World: Proc. 3rd Int. Conf. of Humboldt's Colleagues in Kyrgyzstan), Bishkek, 2010, pp. 83–86.

Biolognesi, C., Genotoxicity of Pesticides: A Review of Human Biomonitoring Studies, *Mutat. Res.*, 2003, no. 6, pp. 251–272.

Dmitriev, S.G., Cytogenetic Instability in Three Rodent Species from the Zone of a Chemical Plant Located in Northern Russia, *Russ. J. Ecol.*, 1997, no. 6, pp. 398–401.

Dubinin, N.P., *Radiatsionnyi i khimicheskii mutagenez: Izbr. trudy* (Radiation and Chemical Mutagenesis: Selected Works), Moscow: Nauka, 2000, vol. 2.

Fung, H.T., Chan, K.K., Ching, W.M., and Kam, C.W., A Case of Accidental Ingestion of Ant Bait Containing Fipronil, *Clin. Toxicol.*, 2003, vol. 41, no. 3, pp. 245–248.

Gavrilov, V.B. and Mishkorudnaya, M.I., Spectrophotometric Determination of Lipid Peroxide Contents in Blood Plasma, *Lab. Delo*, 1983, no. 3, pp. 23–25.

Gileva, E.A., Bol'shakov, V.N., Kosareva, N.L., and Gabitova, A.T., Chromosome Aberration Frequency in Synanthropic House Mice As an Indicator of the Genotoxic Effect of Environmental Pollution, *Dokl. Ross. Akad. Nauk*, 1992, vol. 322, no. 5, pp. 1058–1061.

González, M., Soloneski, S, Reigosa, M.A., and Larramendy, M.L., Effect of Dithiocarbamate Pesticide Zineb and Its Commercial Formulation, Azzurro: 4. DNA Damage and Repair Kinetics Assessed by Single Cell Gel Electrophoresis (SCGE) Assay on Chinese Hamster Ovary (CHO) Cells, *Mutat. Res.*, 2003, vol. 534, nos. 1–2, pp. 145–154.

Grafodatskii, A.S. and Radzhabli, S.I., *Khromosomy sel'skokhozyaistvennykh i laboratornykh mlekopitayushchikh* (Chromosomes of Farm and Laboratory Animals), Novosibirsk: Nauka, 1988.

Losi-Guembarovski, R., Santos, F.V., Dias, F.L., et al., Assessment of the Ability of Imazaquin Herbicide to Induce Chromosomal Aberrations in Vitro in Cultured Chinese Hamster Ovary Cells and Micronuclei in Vivo in Mice, *Food Chem. Toxicol.*, 2004, vol. 42, pp. 1245–1249.

Mukhopadhyay, I., Chowdhuri, Dk., and Bajpayee, M., Evaluation of in Vivo Genotoxicity of Cypermethrin in *Drosophila melanogaster* Using the Alkaline Comet Assay, *Mutagenesis*, 2004, no. 19, no. 2, pp. 85–90.

Opredelenie ostatochnykh kolichestv fipronila i ego metabolita fipronil-sul'fona v vode, pochve, klubnyakh kartofelya, zerne i solome zernovykh kolosovykh kul'tur metodom gazozhidkostnoi khromatografii: Metodich. ukazaniya. MUK 4.1.1400-03 (Determination of Residual Amounts of Fipronil and Its Metabolite, Fipronil-Sulfone, in the Water, Soil, Potato Tubers, and Cereal Grain and Straw by Gas–Liquid Chromatography: Methodological Guidelines MUK 4.1.1400-03), Moscow, 2003.

Rokitskii, N.A., *Vvedenie v statisticheskuyu genetiku* (Introduction to Statistical Genetics), Minsk: Vysheishaya Shkola, 1978.

Sagitov, A.O., Ysak, S., and Evdokimov, N.Ya., Prognosis of the Amount of Chemical Treatment against Harmful Locusts in Kazakhstan, *Zashchita i Karantin Rastenii*, 2002, no. 1, pp. 19–20.

Spravochnik pestitsidov (yadokhimikatov), razreshennykh k primeneniyu na territorii Respubliki Kazakhstan (Reference Book of Pesticides and Toxic Chemicals Authorized for Use in the Republic of Kazakhstan), Almaty: ANES, 2008.

Stal'naya, I.D. and Garishvili, G.G., Determination of Malonic Dialdehyde with Thiobarbituric Acid, in *Sovremennye metody v biokhimii* (Modern methods in Biochemistry), Orekhovich, V.N., Ed., Moscow, 1977, pp. 66–68.

Vremennye metodicheskie ukazaniya po opredeleniyu ostatochnykh kolichestv metabolita fipronil-sul'fona v vode, pochve, klubnyakh kartofelya, zerne i solome zernovykh kolosovykh kul'tur metodom gazozhidkostnoi khromatografii (Provisional Methodological Guidelines for Determining Residual Amounts of Fipronil-Sulfone in the Water, Soil, Potato Tubers, and Cereal Grain and Straw by Gas–Liquid Chromatography), Moscow, 1997.

SPELL: 1. fipronil, 2. phenylpyrazoles